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Donor-specific HLA antibodies in predicting crossmatch outcome: Comparison of three different laboratory techniques

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ABSTRACT

The virtual crossmatch, which is based on single antigen bead technology, is used in the prediction of crossmatch results. However, this assay differs in sensitivity and specificity from crossmatch methods. In our study, the results of physical crossmatches, performed with three different methods, were assessed against virtual crossmatch results. The aim was to determine the potential cut-off values for donor specific antibodies (DSA) that would predict the crossmatch results obtained by different methods. The results of different crossmatch techniques were correlated with the virtual crossmatch. The receiver operating characteristic (ROC) analysis revealed the Flow cytometric crossmatch (FCXM) and Luminex crossmatch (LXM) to be the most accurate, with area under curve (AUC) values of 0.861 and 0.805, respectively. While we found that the virtual crossmatch correlated well with all the crossmatch results, FCXM produced the best results (83% of the DSA detected). LXM outperformed the other tests in terms of the accuracy in separating class II DSA.

1. Introduction

The relevance of preformed human leukocyte antigen (HLA) antibodies in the context of transplantation was discovered in the 1960s [1]. Antibodies detected by Complement-dependent cytotoxicity (CDC) have the capacity to destroy donor cells via complement-mediated cytotoxicity. The fact that these antibodies have a clear effect on the cells attests to the clinical relevance of this assay for both screening and crossmatching [2]. However, accurate identification with CDC is problematic and the method is also very laborious. Innovations in commercial HLA antibody screening kits utilizing bead array technology have enabled the determination of antibody specificities with high accuracy and sensitivity [3,4]. This technology is now widely used and has replaced HLA antibody screening with the CDC method in many laboratories. The development of crossmatch techniques, however, has not made similar progress and consequently there are still many different crossmatch techniques in use today. This has led to a situation where crossmatching and screening are based on completely different technologies, quite often not even measuring the same variable.

As the main idea of a screening program is to predict crossmatches that would be negative, a screening method with differing sensitivity and specificity is far from ideal.

Predetermined antibody specificities allow virtual crossmatching to be performed as soon as the donor candidate has been typed for HLA. A virtual crossmatch performed with single antigen beads correlates with the graft outcome, but the correlation with a physical crossmatch is often not optimal due to differences in assays [5–7].

The predictive value of HLA antibodies identified with single antigen beads differs for each crossmatch method. Since FCXM, similarly to the routine bead array technique, identifies also non-complement-binding antibodies, it could be expected to produce more concordant results than Complement-dependent cytotoxicity crossmatch (CDCXM). However, false positive FCXM results are regularly seen and the crossmatch is not HLA-specific either [6]. One of the most recent crossmatch methods is a solid phase method where donor HLA molecules are captured on beads and analysed with a bead array. This method, LXM, is one of two HLA-specific crossmatch methods available, and it should correlate well with a virtual crossmatch [8]. The other available donor specific crossmatch, which is not evaluated in this study, is based on enzyme-linked immunosorbent assay [9].

2. Objective

Clinical laboratories working with organ transplantation struggle

Abbreviations: AUC, area under curve; CDC, Complement-dependent cytotoxicity; CDCXM, Complement-dependent cytotoxicity crossmatch; DSA, donor specific antibodies; DTT, dithiothreitol; FCXM, Flow cytometric crossmatch; LXM, Luminex crossmatch; MFI, Mean fluorescence intensity; ROC, receiver operating characteristic

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with the fact that antibody screening and the various crossmatching techniques differ greatly in terms of their sensitivity. The aim of this study was to compare the predictive value of the virtual crossmatch with various crossmatch techniques used in clinical laboratory settings and to identify the cut-off values with the best predictability.

3. Materials and methods

3.1. Sample collection

A total of 288 serum samples from 235 patients were used in crossmatching (the latest serum of each patient was used). Samples were stored frozen at − 20 °C. Crossmatching with different techniques was performed against splenocytes of 40 deceased donor candidates. For CDCXM, freshly separated cells were used. For the other crossmatching assays, splenocytes stored in liquid nitrogen were used.

3.2. Donor HLA typing

Donors were HLA typed with the complement-mediated lymphocytotoxicity test (Biotest Rockaway, NJ) and a low-resolution polymerase chain reaction with sequence-specific primers (One Lambda Inc., Canoga Park, CA). Supplementary typing for HLA-A, B, C, DRB1-5, DQA1, DQB1, DPA1 and DPB1 was performed with a polymerase chain reaction with sequence-specific oligonucleotide probes (One Lambda) when the result was needed to confirm the donor-specificity of an antibody.

3.3. HLA antibody screening

Serum samples stored for routine crossmatching were used. Antibodies were screened and identified with Luminex-based commercial kits (LABScreen®, One Lambda) from untreated serum samples. All specificities were identified with single antigen beads with a cut-off value of the baseline normalized value 1000 MFI for an individual bead representing a single DSA. All sera were tested for HLA class I (HLA-A, -B and -Cw) and class II (HLA-DR, -DQ and -DP) antibodies. Antibodies were assigned with the HLA Fusion™ software v.3.2. DSA was determined by comparing assigned antibodies to the serological equivalent of the donor's HLA type. For anti-DQ and –DP antibodies, α and β chain combinations were used in the analysis. Known Finnish haplotypes were utilised when a bead was selected to represent the antigen of the donor [10]. MFI values of the donor-specific antibodies were recorded. The sum of all individual DSAs above 1000 MFI was reported as the cumulative DSA [11,12].

3.4. Complement-dependent cytotoxicity crossmatch

CDCXM was performed during on-call with fresh density gradient-purified donor spleen cells using the technique by Amos [13]. Crossmatching was performed in parallel with various volumes of serum (1 μl, 5 μl, 0.1 μl) as well as with an excess of complement (double amount). Serum and cells were incubated 20 min at room temperature and after complement addition 60 min at room temperature. Reactivity was scored according to the percentage of dead cells with the international workshop scoring: score 1: < 1%; 2: 1–20%; 4: 21–50%; 6: 51–80%; 8: 81–100%. As a local modification, any cell death above background was considered positive (score 2). The highest reactivity of parallel testing was used as the strength of an individual crossmatch in further analysis. All crossmatches were performed without dithiothreitol (DTT).

3.5. Flow cytometric crossmatch

FCXM was performed retrospectively with frozen donor splenocytes [14]. T cell IgG and IgM and B cell IgG crossmatches were performed. In each test, 500,000 splenocytes were incubated with 50 μl of serum for 30 min. After washing, T and B cells were identified with PE-anti-CD3 and PE-anti-CD19 (Cat no. 345765, 345777; BD Biosciences, San Jose, CA). The secondary antibody was a FITC-conjugated $F(ab)_2$ anti-human IgG (Cat no. 109-096-098 Jackson ImmunoResearch, West Grove, PA). After addition of fluorescence-labelled antibodies, cells were incubated for 15 min in the dark. Cells were analysed using FACScan instrument (BD Biosciences). A linear channel shift of at least 40 channels for T cells and 60 channels for B cells was considered positive.

3.6. Luminex crossmatch

LXM with Tepnel Donor specific antibody kit (currently Immucor Lifecodes) was performed retrospectively according to the manufacturer's protocol except for frozen splenocytes. Cells were thawed and washed to remove dimethyl sulfoxide and then lysed. For each test, a lysate containing 2.2×10^6 splenocytes was prepared. The lysate (8 μl) was then incubated with 5 μl of capture beads coated with either anti-HLA class I or class II for 30 min in the dark at room temperature. These beads with captured donor HLA molecules where then incubated with 12 μl of the patient serum for 30 min at room temperature in the dark. Binding of anti-HLA antibodies was detected with anti-human IgG conjugated with PE (R-phycoerythrin). Samples were run on LabScan 200 and analysed with the LifeMatch software (Tepnel, Lifecodes). A positive control bead (IgG) was used to verify the binding of anti-IgG-PE. Three negative control beads were included and MFI values above 1000 against all three negative control values were considered positive.

3.7. Statistical analysis

Categorical variables were analysed with the Fisher's exact test. In the ROC analysis, also DSA values below 1000 MFI were included as the analysis was used to determine the best cut-off value. The sensitivity and specificity of the various crossmatching methods were assessed with the ROC analysis. The accuracy of the test was classified as the AUC with 0.9–1: excellent; 0.8–0.9: good; 0.7–0.8: fair; 0.6–0.07: poor; 0.5–0.06: fail. Results with a P value of ≤ 0.05 were considered statistically significant. Analyses were performed with the SPSS statistics version 21.0 software (IBM, Armonk, NY, USA).

4. Results

4.1. HLA antibody profile

Of the 364 crossmatches performed, the majority (68% (246)) were performed with serum without HLA antibodies. Only 23% (83) of the crossmatches were performed against DSA. The mean cumulative DSA in DSA-positive crossmatches was 25,000 ± 23,000 MFI, with the highest identified value being 90,000 MFI. Class I DSA was detected in 10% (37) and class II DSA in 5% (17) of the crossmatches, while both class I and II DSA were detected in 8% (29) of the crossmatches. Antibodies were most commonly directed against donor HLA-B antigens (13% (48)) and least frequently against HLA-DRB3–5 (5% (17)) and HLA-DP (5% (17)) (Fig. 1).

4.2. Complement-dependent cytotoxicity crossmatches

Crossmatches were performed against donor splenocytes containing both T and B cells showing expression of Class I and Class II HLA antigens. A total of 30% (111/364) of the crossmatches were positive, with the highest scores being 2 (26%), 4 (35%), 6 (17%) and 8 (22%). DSA was present in only 50% (55/111) of the positive CDCXM (Table 1). A total of 11% (28/253) of the negative crossmatches were performed against DSA. The score for cell death in CDCXM correlated well with the degree of DSA positivity: for positive crossmatches with weak positivity (score 2) only 31% of the crossmatches were against